Notice of Allowability	Application No.	Applicant(s)	
	10/031,918	NOEL ET AL.	
	Examiner	Art Unit	
	Nashaat T. Nashed, Ph. D.	1656	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *the amendment filed 11/2/06*.

2. ☒ The allowed claim(s) is/are *17, 23, 26, and 40-56*.

3. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
   a) ☐ All b) ☐ Some* c) ☐ None of the:
      1. ☐ Certified copies of the priority documents have been received.
      2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
      3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

4. ☐ A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   (a) ☐ including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached
      1) ☐ hereto or 2) ☐ to Paper No./Mail Date _____.
   (b) ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Notice of Draftperson's Patent Drawing Review (PTO-948)
3. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____
4. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
5. ☐ Notice of Informal Patent Application
6. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____ .
7. ☒ Examiner's Amendment/Comment
8. ☐ Examiner's Statement of Reasons for Allowance
9. ☐ Other _____.

The application has been amended as requested in the communication filed November 2, 2006. Accordingly, claim 17, 40, 43, 44, and 45 have been amended.

Claims 17, 23, 26, and 40-56 are pending and under consideration.

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with Stephen E. Reiter on December 1, 2006.

The application has been amended as follows:

Amend the specification as shown below:

(1) Please amend page 23, line 25 as follows:

TABLE 3 (SEQ ID NO:[[__]]1)

(2) Please amend the paragraph starting at page 4, line 13 as follows:

Figure 5A shows the CHS-naringenin complex viewed down the CoA-binding tunnel. The ribbon diagram at the top left has been rotated 90 degrees around the y-axis from the orientation shown in **Figure 2A**. This view approximates the global orientation of the CHS dimer used for the close-up view of the naringenin binding site depicted in stereo. Again, the black box highlights the region of CHS shown in stereo close-up. Hydrogen bonds are depicted as dashed cylinders. **Figure 5B** illustrates a comparison of the CHS apoenzyme, CHS-naringenin, and CHS-resveratrol structures. Protein backbone atoms for the three refined structures (apoenzyme, naringenin, and resveratrol) were superimposed by least squares fit in O. The position of bound naringenin and resveratrol are shown. For reference, a modeled low energy conformation of chalcone is indicated by dashed cylinders. Strands β1d and β2d for each complex are also depicted (see **Figure 3**). β2d does not change in all the complexes examined, but β1d moves in the CHS-resveratrol complex. **Figure 5C** presents representative sequence alignment of the β1d-β2d region is given with positions 255,266, and 268 **(SEQ ID NO:1)** highlighted. The first three sequences **(SEQ ID NOs:2-4)** follow a CHS-like cyclization pathway, while the last three **(SEQ ID NOs:5-7)** use the STS-cyclization pathway. Figure prepared with MOLSCRIPT and rendered with POV-Ray.

(3) Please amend the paragraph starting at page 9, line 23 as follows:

"Nonmutated synthase" includes a synthase wherein no R-group(s) are changed relative to the active site of CHS (see, for example, PDB Accession No. 1BI5). A nonmutated synthase according to the present invention may or may not have amino acid residues outside of the active site that are the same as those taught for native CHS **(SEQ ID NO:1)**. In addition, a nonmutated synthase is a synthase having an active site comprising α-carbons having the coordinates as given in Table 1 and having the arrangements of R-groups associated with □-carbons as given in Table 1.

(4) Please amend the paragraph starting at page 23, line 1 as follows:

The invention also relates to structural coordinates of said polyketide synthases, use of said structural coordinates to develop structural information related to polyketide synthase homologues, mutants, and the like, and to crystal forms of such synthases. Furthermore, the invention, as disclosed herein, provides a method whereby said α-carbon structural coordinates specifically determined for atoms comprising the active site of said synthase, as shown in Table 1 and including C164, H303, and N336 **(SEQ ID NO:1)**, can be used to develop synthases wherein R-groups associated with active site α-carbon atoms are different from the R-groups found in native CHS **(SEQ ID NO:1)**, *e.g.*, are mutant synthases. In addition, the present invention provides for production of mutant polyketide synthases based on the structural information provided herein and for use of said mutant synthases to make a variety of polyketide compounds using a variety of substrates.

(5) Please amend the paragraph starting at page 148, line 10 as follows:

The crystals of the present invention belong to the tetragonal space group. The unit cell dimensions vary by a few angstroms between crystals but on average, chalcone synthase native **(SEQ ID NO:1)** crystals belong to space group $P3_221$ with unit cell dimensions of a = b = 97.54 Å; c = 65.52 Å, $\alpha = \beta =$ 90°, γ = 120° with a single monomer per asymmetric unit. Stilbene synthase crystals belong to space group C222 with unit cell dimensions of a = 74.94 Å, b = 86.63 Å, c = 364.18 Å, $\alpha = \beta = \gamma$ = 90°. Pyrone synthase crystals belong to space group P3121 with unit cell dimensions of a = 82.15 Å, b = 241.33 Å, $\alpha = \beta$ = 90°, γ = 120° with one PS dimer per asymmetric unit.

(6) Please amend the paragraph starting at page 150, line 19 as follows:

Mutant proteins of the present invention may be prepared in a number of ways available to the skilled artisan. For example, the gene encoding wild-type CHS **(SEQ ID NO:1)** may be mutated at those sites identified herein as corresponding to amino acid residues identified in the active site by means currently available to the artisan skilled in molecular biology techniques. Said techniques include oligonucleotide-directed mutagenesis, deletion, chemical mutagenesis, and the like. The protein encoded by the mutant gene is then produced by expressing the gene in, for example, a bacterial or plant expression system.

(7) Please amend the paragraph starting at page 175, line 14 as follows:

Crystallization. CHS crystals (wild-type and $C_{164}S$ mutant, **SEQ ID NO:1**) were grown by vapor diffusion at 4° C in 2 µ1 drops containing a 1:1 mixture of 25 mg/ml protein and crystallization buffer (2.2-2.4 M ammonium sulfate and 0.1 M PIPES, pH 6.5) in the presence or absence of 5 mM DTT. Prior to freezing at 105° K, crystals were stabilized in 40% (v/v) PEG400,0.1 M PIPES (pH 6.5), and 0.050-0.075 M ammonium sulfate. This cryoprotectant was used for heavy atom soaks. Likewise, all substrate and product analog complexes were obtained by soaking crystals in cryoprotectant containing 10-20 mM of the compound.

(8) Please amend the paragraph starting at page 175, line 22 as follows:

Data Collection and Processing. X-ray diffraction data were collected at 105° K using a DIP2000 imaging plate system (Mac-Science Corporation, Japan) and CuK radiation produced by a rotating anode operated at 45 kV and 100 mA and equipped with double focusing Pt/Ni coated mirrors. Native CHS **(SEQ ID NO:1)** belong to space group $P3_2,21$ with unit cell dimensions of $a = b =$ 97.54Å; c = 65.52 Å with a single monomer per asymmetric unit. Data were indexed and integrated using DENZO (Otwinowski & Minor, Meth. Enzymol. 276:307-326, 1997) and scaled with SCALEPACK (Otwinowski & Minor, Meth. Enzymol. 276:307-326, 1997). The heavy atom derivative datasets were scaled against the native dataset with SCALEIT (CCP4 Suite: Programs for protein crystallography, Acta Crystallogr. D 50:760-763, 1994).

(9) Please amend the paragraph starting at page 176, line 4 as follows:

Structure determination. MIRAS was used to solve the structure of native CHS **(SEQ ID NO:1)** using native data set 1 (1.8 Å). Initial phasing was performed with derivative datasets including reflections to 2.3A resolution. Heavy atom positions for the Hg (OAc) 2 derivative were estimated by inspection

of difference Patterson maps using the program XTALVIEW (McRee, J. Mol. Graph. 10:44-46,1992) and initially refined with MLPHARE (Otwinowski, Z. in CCP4 Proc. *80-88*, Daresbury Laboratory, Warrington, UK, 1991). Heavy atom positions for the additional derivative data sets were determined by difference Fourier analysis using phases calculated from the Hg $(OAc)_2$ data set and the Hg positions. These sites were confirmed by inspection of difference Patterson maps. Final refinement of heavy atom parameters, identification of minor heavy atom binding sites, and phase-angle calculations were performed with the program SHARP (de La Fortelle, & Bricogne, Meth. Enzymol. 276:472-494,1997). MIRAS phases were improved and extended to 1.8 Å by solvent flipping using the CCP4 program SOLOMON (Abrahams, & Leslie, Acta Crystallogr. D 52: 30-42,1996).

(10) Please amend the paragraph starting at page 177, line 7 as follows:

Recombinant alfalfa CHS2 was expressed in *E. coli*, affinity purified using an N-terminal poly-His linker, and crystallized. The structure of wild-type CHS **(SEQ ID NO:1)** was determined using multiple isomorphous replacement supplemented with anomalous scattering (MIRAS) (Table X). The final 1.56 Å resolution apoenzyme model of CHS included 2982 protein atoms and 355 water molecules. In addition, the structures of a series of complexes were obtained by difference Fourier analysis. First, a crystal of a mutant (Cl64S) was soaked with malonyl-CoA. This mutant retains limited catalytic activity, and the resulting acetyl-CoA complex yields insight on the decarboxylation reaction. The same mutant was also complexed with hexanoyl-CoA to mimic the structure of a linear polyketide-CoA reaction intermediate. Finally, two product analogs, naringenin and resveratrol (see Figure 1) were complexed with CHS to provide information on how the enzyme governs sequential addition of acetates to the coumaroyl moiety and how CHS controls the stereochemistry of the polyketide cyclization reaction. In plants, chalcone isomerase rapidly and stereospecifically converts chalcone to naringenin ((-)(2S)-5,7,4'-trihydroxyflavanone) through an additional ring closure. This reaction also occurs at a slower rate and non-stereospecifically in solution. As such, naringenin provides a suitable mimic of the CHS reaction product. Finally, since STS uses the same substrates as CHS but a different cyclization pathway for the biosynthesis of resveratrol, resveratrol was also soaked into CHS to investigate the structural features governing cyclization of the same substrates into two different products.

(11) Please amend the paragraph starting at page 189, line 24 as follows:

The x-ray crystal structures of 2-PS and CHS imply that the size of the active site cavity limits polyketide length and modulates folding of the polyketide chain. Wild-type CHS**(SEQ ID NO:1)** generates the tetraketide chalcone and 2-

PS produces the triketide methylpyrone. Likewise, the CHS I254M mutant **(SEQ ID NO:1)** also yields chalcone. Interestingly, the T197L, G256L, and S338I mutants **(SEQ ID NO:1)** do not form chalcone. Crystallographic analysis of the G256L and S338I mutants **(SEQ ID NO:1)** demonstrates that the substituted sidechains adopt conformations similar to the corresponding residues in 2-PS without altering the position of the protein backbone. Since the T197L, G256L, and S338I mutants altered product formation, a CHS triple mutant was generated. Consistent with the proposal that cavity volume dictates polyketide length, the T197L/G256L/S338I mutant produces only methylpyrone, as confirmed by liquid chromatography/mass spectroscopy (LC/MS). LC/MS/MS analysis was performed by the Mass Spectroscopy facility of the Scripps Research Institute. Scaled-up assays (2 ml reaction volume) with the CHS T197L/G256L/S338I mutant and 2-PS were performed. Extracts were analyzed on a Hewlett-Packard HP1100 MSD single quadrupole mass spectrometer coupled to a ZorbaxSB-C_{18} column (5 µm, 2.1 mm x 150 mm). HPLC conditions were as follows: gradient system from 0 to 100% methanol in water (each containing 0.2% acetic acid) within 10 min; flow rate 0.25 ml min^{-1}. LC/MS/MS data from both reactions were identical: 6-methyl-4-hydroxy-2pyrone, R_t = 5.068 min; $[M-H]^-$125 (41); $[M-H-CO_2]^-$81 (100). The numbers show *m/z* values with relative intensities in parenthesis. The observed fragmentation matches previously published data.

(12) Please amend the paragraph starting at page 191, line 8 as follows:

Functional diversity among otherhomodimeric iterative PKSs, like *p*-coumaroyltriacetic acid synthase (CTAS), acridone synthase (ACS), and the *rppA* protein from *Streptomyces griseus*, likely results from variations of residues lining the initiation/elongation cavity. As demonstrated, positions 197, 256, and 338 of SEQ ID NO:1 distinguish between tetraketide products derived from a final Claisen condensation in wild-type CHS **(SEQ ID NO:1)** and triketide products derived from an enolate-directed condensation in the CHS triple mutant. Although CHS, CTAS, and ACS generate tetraketides, each enzyme differs in either the cyclization reaction or in the identity of the starter molecule. CTAS forms the same enzyme-bound tetraketide as CHS but does not catalyze the final cyclization reaction. Comparison of these two enzymes reveals that substitution of Thr 197 of SEQ ID NO:1 in CHS with an asparagine in CTAS may prevent the covalently-bound tetraketide intermediate from undergoing cyclization into chalcone. ACS uses N-methylanthranoyl-CoA as a starting substrate to produce the alkaloid acridone. Three differences between CHS (Thr_{132}, Ser_{133}, and Phe_{265} of SEQ ID NO:1) and ACS (Ser_{132}, Ala_{133}, and Val_{265}) may alter starter molecule specificity. In ACS, these changes likely widen the portion of the cavity corresponding to the *p*-coumaroyl-binding site in CHS to accommodate N-methylanthranoyl-CoA binding. Comparative changes in the active site cavity

allow formation of longer polyketides. The *rppA* protein forms a pentaketide from five acetates derived from malonyl-CoA decarboxylation. Thr_{137}, Ala_{138}, Thr_{199}, Leu_{202}, Met_{259}, Leu_{261}, Leu_{268}, Pro_{304}, and Ile_{343} of 2-PS are replaced by Cys_{106}, Thr_{107}, Cys_{168}, Cys_{171}, Ile_{228}, Tyr_{230}, Phe_{237}, Ala_{261}, and Ala_{295}, respectively, in the *rppA* protein. Models of the *rppA* protein based on the 2-PS and CHS structures show that cavity volume is 1145 $Å^3$ in the *rppA* protein versus 274 $Å^3$ in 2-PS (or 923 $Å^3$ in CHS). Manipulation of the active site through amino acid substitutions offers a strategy for increasing the molecular diversity of polyketide formation through both the choice of starter molecule and the number of subsequent condensation steps.

Claims 17, 23, 26, and 40-56 are allowed.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Nashaat T. Nashed, Ph. D. whose telephone number is 571-272-0934. The examiner can normally be reached on MTWTF.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Kathleen M. Kerr can be reached on 571-272-0931. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

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Art Unit 1656